LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AB The present invention described herein is directed to the use of random genetic mutation of a cell to produce novel antibiotics by blocking the endogenous mismatch repair activity of a host cell by introducing a dominant neg. mismatch repair (MMR) gene such as **PMS2** (preferably human **PMS2**), MLH1, PMS1, MSH2, or MSH3. The cell can be a mammalian cell that produces an antimicrobial agent naturally, or a cell that is placed under selective pressure to obtain a novel antimicrobial mol. that attacks a specific microbe. Moreover, the invention describes methods for obtaining enhanced antimicrobial activity of a cell line that produces an antimicrobial activity due to recombinant expression or as part of the innate capacity of the cell to harbor such activity. An embodiment of the invention described herein is directed to the creation of genetically altered host cells with novel and/or increased antimicrobial prodn. that are generated by a method that interferes with the highly ubiquitous and phylogenetically conserved process of mismatch repair. An example of a dominant neg. allele of a mismatch repair gene is the human gene hPMS2-134, which carries a truncation mutation at codon 134. The mutation causes the product of this gene to abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide contg. the N-terminal 133 amino acids. Such a mutation causes an increase in the rate of mutations that accumulate in cells after DNA replication. Syrian Hamster TK fibroblasts transfected with a mammalian expression vector contg. a novel antimicrobial polypeptide called mlg1 and grown in the presence of Bacillus subtilis were able to suppress the growth of the microbes. Escherichia coli bacterial growth was significantly suppressed in TK-ts13 cells constitutively expressing the dominant-neg. mismatch repair gene, TK-hPMS2-134.

RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2002 ACS
AN 2002:368239 CAPLUS
DN 136:364875
TI Generating hypermutable antibody-producing cells using **dominant negative** alleles of mismatch repair genes
IN Nicolaides, Nicholas C.; Grasso, Luigi; Sass, Philip M.
PA Morphotek Inc., USA
SO PCT Int. Appl., 75 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2002037967 | A1 | 20020516 | WO 2000-US30588 | 20001107 |

PI WO 2002037967 A1 20020516
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AB The invention described herein is directed to the use of random genetic mutation throughout an antibody structure in vivo by blocking the

endogenous mismatch repair (MMR) activity of a host cell producing Igs that encode biochem. active antibodies. The invention also relates to methods for repeated in vivo genetic alterations and selection for antibodies with enhanced binding and pharmacokinetic profiles. The invention also provides methods of making hypermutable antibody producing cells by introducing a dominant neg. mismatch repair (MMR) gene such as **PMS2** (preferably human **PMS2**), MLH1, PMS1, MSH2, or MSH2 into cells that are capable of producing antibodies. The dominant neg. allele of a mismatch repair gene may be a truncation mutation of a mismatch repair gene (preferably a truncation mutation at codon 134, or a thymidine at nucleotide 424 of wildtype **PMS2**). The invention also provides methods in which mismatch repair gene activity is suppressed. This may be accomplished, for example, using antisense mols. directed against the mismatch repair gene or transcripts. These methods are useful for generating genetic diversity within Ig genes directed against an antigen of interest to produce altered antibodies with enhanced biochem. activity or increased level of antibody prodn. The enhanced rate of mutation can be further augmented using mutagens. The invention demonstrated that a germline truncating mutation in human gene **PMS2** at codon 134 could exert a dominant neg. effect, resulting in biochem. and genetic manifestations of mismatch repair (MMR) deficiency. The invention also demonstrated that dominant neg. mismatch repair gene alleles cause a defect in MMR activity. The invention further demonstrated that MMR and genetic stability can be restored by expressing a MMR gene complementing gene.

RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2002 ACS
AN 2001:851428 CAPLUS
DN 136:1565
TI A method for generating hypermutable cells using **dominant negative** alleles of mismatch repair genes
IN Nicolaides, Nicholas C.; Sass, Philip M.; Grasso, Luigi; Vogelstein, Bert; Kinzler, Kenneth W.
PA The Johns Hopkins University, USA; Morphotek Inc.
SO PCT Int. Appl., 59 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| PI WO 2001088192 | A2 | 20011122 | WO 2001-US15376 | 20010514 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| US 2002055106 | A1 | 20020509 | US 2001-853646 | 20010514 |
| PRAI US 2000-203905P | P | 20000511 | | |
| US 2000-204769P | P | 20000517 | | |

AB Dominant neg. alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. By introducing these genes into cells and transgenic animals, new cell lines and animal varieties with novel and useful properties can be prepd. more efficiently than by relying on the natural rate of mutation. The enhanced rate of mutation can be further augmented using mutagens. Moreover, the hypermutability of

mismatch repair deficient cells can be remedied to stabilize cells or mammals with useful mutations. The invention demonstrated that a germline truncating mutation in human gene **PMS2** at codon 134 could exert a dominant neg. effect, resulting in biochem. and genetic manifestations of mismatch repair (MMR) deficiency. The invention also demonstrated that dominant neg. mismatch repair gene alleles cause a defect in MMR activity. The invention further demonstrated that MMR and genetic stability can be restored by expressing a MMR gene complementing gene.

L4 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2002 ACS
AN 2001:598157 CAPLUS
DN 135:176403
TI Generation of hypermutable microbes using **dominant negative** alleles of mismatch repair protein genes
IN Nicolaides, Nicholas C.; Sass, Philip M.; Grasso, Luigi; Vogelstein, Bert; Kinzler, Kenneth W.
PA The Johns Hopkins University, USA
SO PCT Int. Appl., 68 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2001059092 | A2 | 20010816 | WO 2001-US4339 | 20010212 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| US 2002068284 | A1 | 20020606 | US 2001-780675 | 20010212 |
| PRAI US 2000-181929P | P | 20000211 | | |

AB Bacteria are manipulated to create desirable output traits using dominant neg. alleles of mismatch repair proteins. Endogeneous mismatch repair (MMR) activity of the host is decreased by use of dominant neg. MMR genes followed by placing the cells under selection to obtain a desired, sought after output trait. Bacterial MMR activity is shown to be inactivated by expression vectors for human **PMS2**-related gene (hPMSR3), human PMS123 gene, and a truncated **PMS2** homolog from Arabidopsis thaliana. Thus, enhanced hypermutation is achieved by a combination of MMR deficiency and exogenously applied mutagens or radiation. MMR activity is restored to the hypermutable bacterial host following strain selection of the variant of interest as a means to genetically "fix" the new mutations in the host genome. Desired traits for com. applications include recombinant manuf. by fermn., biotransformation of substrates, and bioremediation. Stable bacteria contg. desirable output traits are obtained by restoring mismatch repair activity to the bacteria.

L4 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2002 ACS
AN 2000:802345 CAPLUS
DN 133:359757
TI Generation of hypermutable organisms using **dominant negative** alleles of the mismatch repair gene **PMS2**
IN Nicolaides, Nicholas; Vogelstein, Bert; Kinzler, Kenneth W.
PA The Johns Hopkins University, USA
SO U.S., 21 pp.
CODEN: USXXAM
DT Patent

LA English
FAN.CNT 1

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|---|
| PI | US 6146894 | A | 20001114 | US 1998-59461 | 19980414 |

AB Dominant neg. alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. Thus, truncation mutations can be introduced into human wild-type mismatch repair gene **PMS2** at codons 134 or 424 to produce dominant neg. proteins, resulting in hypermutability. The C-terminal region of **PMS2** protein is shown to mediate interaction between **PMS2** and MLH1 (a mutL homolog involved in the mismatch repair process). By introducing these genes into cells and transgenic animals, new cell lines and animal varieties with novel and useful properties can be prepd. more efficiently than by relying on the natural rate of mutation.

RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 6 OF 8 MEDLINE DUPLICATE 1
AN 2000082804 MEDLINE
DN 20082804 PubMed ID: 10615123
TI MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability.
AU Lipkin S M; Wang V; Jacoby R; Banerjee-Basu S; Baxevanis A D; Lynch H T; Elliott R M; Collins F S
CS Genetics Branch, National Human Genome Research Institute, Bethesda, Maryland, USA.
NC CA62225 (NCI)
SO NATURE GENETICS, (2000 Jan) 24 (1) 27-35.
Journal code: 9216904. ISSN: 1061-4036.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF195657; GENBANK-AF195658; GENBANK-AL031135; GENBANK-P14242; GENBANK-P49850; GENBANK-P54277; GENBANK-P54278; GENBANK-Z73520; GENBANK-Z92813
EM 200002
ED Entered STN: 20000218
Last Updated on STN: 20000218
Entered Medline: 20000210

AB DNA mismatch repair is important because of its role in maintaining genomic integrity and its association with hereditary non-polyposis colon cancer (HNPCC). To identify new human mismatch repair proteins, we probed nuclear extracts with the conserved carboxy-terminal MLH1 interaction domain. Here we describe the cloning and complete genomic sequence of MLH3, which encodes a new DNA mismatch repair protein that interacts with MLH1. MLH3 is more similar to mismatch repair proteins from yeast, plants, worms and bacteria than to any known mammalian protein, suggesting that its conserved sequence may confer unique functions in mice and humans. Cells in culture stably expressing a **dominant-negative** MLH3 protein exhibit microsatellite instability. Mlh3 is highly expressed in gastrointestinal epithelium and physically maps to the mouse complex trait locus colon cancer susceptibility I (Ccs1). Although we were unable to identify a mutation in the protein-coding region of Mlh3 in the susceptible mouse strain, colon tumours from congenic Ccs1 mice exhibit microsatellite instability. Functional redundancy among Mlh3, Pms1 and **Pms2** may explain why neither Pms1 nor **Pms2** mutant mice develop colon cancer, and why PMS1 and **PMS2** mutations are only rarely found in HNPCC families.

L4 ANSWER 7 OF 8 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI

AN 2000-04644 BIOTECHDS
TI Generating hypermutable cells for research in hereditary nonpolyposis colorectal cancer syndrome comprises introduction of polynucleotides having a **dominant negative** allele of a mismatch repair gene;
transgenic animal construction by mutagenesis involving introducing mismatch repair gene
AU Nicolaides N; Vogelstein B; Kinzler K W
PA Univ.Johns-Hopkins
LO USA.
PI CA 2240609 14 Oct 1999
AI CA 1998-2240609 11 Aug 1998
PRAI US 1998-59461 14 Apr 1998
DT Patent
LA English
OS WPI: 2000-137587 [13]
AB Creating a hypermutable cell involves introducing into a mammal cell a polynucleotide having a **dominant negative** allele of a mismatch repair gene (MRG) (human **PMS2**, MLH1, PMS1 or MSH2) making the cell hypermutable. Also claimed are: a homogeneous composition of cultured, hypermutable, mammal cells which comprise a **dominant negative** allele of a MRG; a hypermutable mammal transgenic animal with at least half its cells containing a **dominant negative** allele of a MRG; generating a mutation in a gene of interest by growing a mammal cell containing the gene and a **dominant negative** allele of a MRG (where the cell is hypermutable), and testing the cell to see if the gene of interest harbors a mutation; and generating a mutation in a gene of interest by growing a mammal containing the gene of interest and a polynucleotide encoding a **dominant negative** allele of a MRG, and testing the mammal to determine whether the gene of interest harbors a mutation. The method allows new cell lines and animal varieties with new and useful properties to be prepared efficiently. The methods will aid research into hereditary nonpolyposis colorectal cancer syndrome in patients. (50pp)

L4 ANSWER 8 OF 8 MEDLINE DUPLICATE 2
AN 1998032543 MEDLINE
DN 98032543 PubMed ID: 9365839
TI Mechanisms underlying mismatch repair deficiencies in normal cells.
AU Moliaka Y K; Cella M; Chudina A P; Kolesnikova T N; Terracciano L; Cathomas G; Bochkov N P; Buerstedde J M
CS Department of Medical Genetics, Sechenov Moscow Medical Academy, Moscow, Russia.
SO GENES, CHROMOSOMES AND CANCER, (1997 Nov) 20 (3) 305-9.
Journal code: 9007329. ISSN: 1045-2257.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199801
ED Entered STN: 19980122
Last Updated on STN: 20020420
Entered Medline: 19980108
AB Hereditary nonpolyposis colon cancer (HNPCC) is an autosomal dominantly inherited cancer predisposition which is linked to heterozygous mutations in mismatch repair genes. HNPCC tumour cells, in which the remaining wild-type copy of the mismatch repair gene is inactivated, display instability of microsatellite markers reflecting a defect in mismatch repair. Recently, patients carrying either one of two distinct germline mutations in the MLH1 and **PMS2** genes were reported to accumulate somatic mutations of microsatellites in untransformed cells. One of the

mechanisms that might account for this phenomenon was a **dominant negative** effect of the mutant allele. To evaluate this possibility, we examined a different family carrying one of the mutations (deletion of codon 618K in the MLH1 gene) which has been suspected to induce genetic instability in untransformed cells. No mutations in dinucleotide repeat markers were observed in a large number of lymphoblast clones derived from a carrier. Evidence for the deletion of the wild-type allele in two different tumours suggested that the inactivation of both gene copies was required for tumour initiation. These results indicate that the MLH1 618K deletion mutation alone does not necessarily cause marked mismatch repair deficiency in the presence of a wild-type allele.

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US 097074680LP1

Creation date: 09-04-2003
Indexing Officer: NNGUYEN7 - NAM NGUYEN
Team: OIPEBackFileIndexing
Dossier: 09707468

Legal Date: 07-18-2002

| No. | Doccode | Number of pages |
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| 1 | CTNF | 14 |
| 2 | 1449 | 16 |
| 3 | 892 | 3 |

Total number of pages: 33

Remarks:

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